

BLOOD FLOW IN EXPERIMENTAL LIVER TUMORS: EFFECT OF VASOACTIVE DRUGS

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Abstract. The effects of infused glucagon, histamine and vasopressin on blood flow in anaesthetized rats with intrahepatic tumors were studied using microspheres labeled with ⁹⁹Tc^m or ⁵¹Cr isotopes. Considerable circulatory effects were noted both in central hemodynamic parameters as well as in organ and tissue blood flows. Glucagon infusion increased blood flow in the spleen and small intestine while hepatic artery flow was unchanged. Histamine induced a decrease in hepatic and pulmonary blood flow. Vasopressin showed a pronounced decrease in blood flow in all organs measured. Relative tumor blood flow was registered as the ratio between tumor flow and arterial hepatic flow. A relative decrease of tumor blood flow in relation to surrounding liver tissue blood flow was registered after infusion of vasopressin. No effects were seen after glucagon or histamine infusion.

The aim of regional cytostatic infusion therapy is to deliver a high concentration of the drug selectively to the tumor and a low concentration to the general system as a result of the subsequent dilution effect. This is achieved when treating metastatic or primary cancer of the liver (Sundqvist et al., 1978). This model has also been adopted for cancer in other regions (Hafström et al., 1979a). The results of infusion treatment have not been proved to be as effective as expected and no clinical, randomized trial has resulted in increased survival compared with standard intravenous therapy (Ong et al., 1975).

Various drugs have been investigated experimentally with the view to increase the tumor blood flow in relation to normal tissue blood flow. These studies indicate that noradrenaline infusion selectively increases the specific blood flow through hepatic experimental tumors. There were also performed studies of noradrenaline contents of tumor and normal liver tissue as well as qualitative studies of catecholamines with fluorescence microscopy. It was shown that catecholamines were absent in tumors (Hafström et al., 1979b). Tumor vessels

lack smooth muscles and nerves (Krylova, 1969) and thus the reaction of tumor vessels to adrenergic drugs differs from that of normal tissues.

Other vasoactive agents which do not act primarily as adrenergic stimulants on nerves or vessels may affect the relation between tumor and surrounding tissue blood flow.

The aim of the present study was to investigate the effects of histamine, vasopressin and glucagon on blood flow in rat liver tumors in relation to those on blood flow in surrounding normal liver parenchyma measured by double-isotope labelled microspheres using the reference organ method. These drugs were selected because of their pronounced influence on the blood flow in the gastrointestinal tract, particularly the liver.

MATERIAL

The experiments were carried out on 35 male Wistar and Lister rats weighing 229 ± 6 g. Ten–twelve days before the experiment the rats were inoculated in the liver with an experimental adenocarcinoma cell suspension (NG-W) or hepatoma cell suspension (Hep-H) containing 0.5×10^6 cells. This instituted tumor in the liver about 10 mm in size.

Seven rats in a control group were infused with physiological saline at a rate of $0.2 \text{ ml} \cdot \text{min}^{-1}$ (series A), nine rats with glucagon (Novo Industries, Denmark) $4 \text{ micrograms} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ bodyweight (series B), nine rats with histamine (Vitrum AB, Sweden) at a rate of $2.0 \text{ micrograms} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ bodyweight (series C) and ten rats with Postaction® (Ferring AB, Sweden) a synthetic vasopressin $0.04 \text{ units} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ bodyweight (series D) (Table I).

METHODS

The rats were anaesthetized with sodium pentobarbital ($30 \text{ mg} \cdot \text{kg}^{-1}$ bodyweight intraperitoneally) and catheters with

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Table I. Vasoactive drug infusion studies

Series	Drug	Strain	Number	Tumor	Tumor weight, g
A ₁	Saline	Wistar	4	NG-W	0.96±0.12
A ₂	Saline	Lister	3	Hep-H	0.41±0.05
B ₁	Glucagon	Wistar	4	NG-W	0.78±0.20
B ₂	Glucagon	Lister	5	Hep-H	0.25±0.07
C ₁	Histamine	Wistar	5	NG-W	1.35±0.18
C ₂	Histamine	Lister	4	Hep-H	1.02±0.36
D	Vasopressin	Lister	10	Hep-H	1.13±0.22

an outer diameter of 0.63 mm were introduced: one into the external jugular vein for infusion of drugs, one via the left femoral artery into the lower abdominal aorta for drawing a reference blood sample and one via the right carotid artery into the left ventricle of the heart for injection of the microspheres. The Harvard pump suctioning the reference blood sample was started just prior to injection of the microspheres. Continuous registration of heart rate and blood pressure was carried out throughout the experiment (for details see Hafström et al., 1979). Tracer Sephadex microspheres (15 μ m) were used, labelled with $^{99}\text{Tc}^m$ or ^{51}Cr isotopes. Each injection contained 800 000–1 000 000 spheres suspended in 0.2 ml Ficoll 70® solution.

After the injection of the $^{99}\text{Tc}^m$ labelled microspheres, the rats were infused with saline or the vasoactive drug. When circulation had reached a steady state registered by blood pressure and heart rate, the injection of the ^{51}Cr labelled microspheres took place. Then the rats were killed, specimens removed, weighed and placed into plastic tubes and counted together with the reference samples in a

two-channel well-type scintillation counter. Blood flow was calculated using a computerized program especially designed for this experiment. The ratio between tumor and surrounding liver arterial blood flow was calculated in order to establish relative tumor blood flow.

Student's t-test based on paired observations was used for the statistical analyses.

RESULTS

All values are given as mean±S.E.M. The results from the two strains of rats, Wistar and Lister, in each experimental series are analysed both separately and together. The results are given for the two strains together but when the differences between the two strains are prevalent, these are notified.

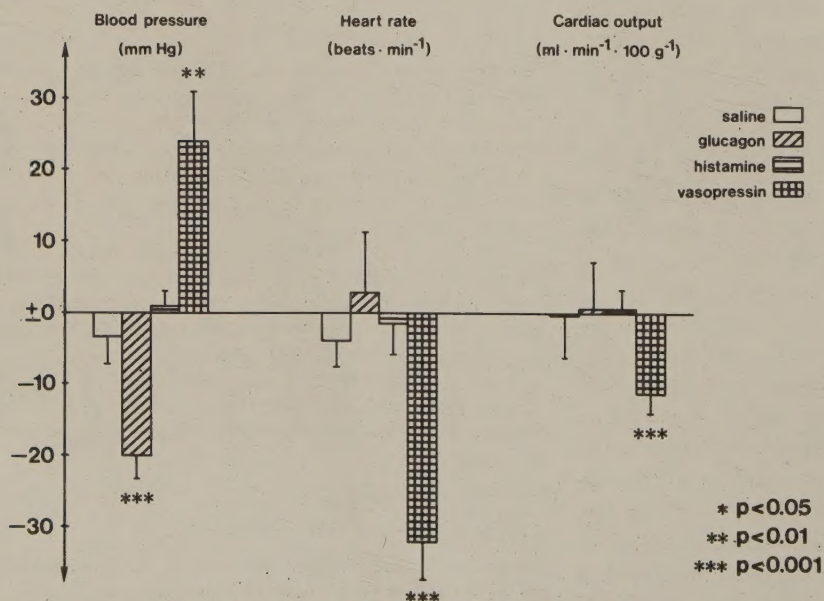


Fig. 1. Changes in blood pressure, heart rate and cardiac output after infusion of saline, glucagon, histamine and

vasopressin. The bars indicate the mean differences ±S.E.M. based on paired observation in each group.

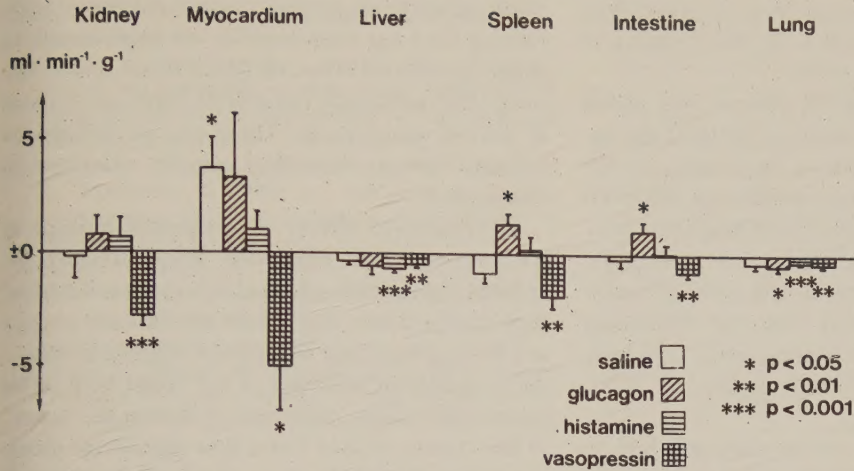


Fig. 2. Changes in organ blood flow after infusion of saline, glucagon, histamine and vasopressin. The bars in-

dicates mean differences $\pm$ S.E.M. based on paired observations.

The effects of infusion on blood pressure, heart rate and cardiac output are summarized in Fig. 1. Saline infusion induced no changes in blood pressure, heart rate or cardiac output. Glucagon infusion caused a significant decrease in blood pressure but had no effect on heart rate or cardiac output. Histamine gave no changes. Vasopressin produced a significant increase in blood pressure and a decrease in heart rate and in cardiac output.

The effects of organ blood flow after infusion of the drugs are shown in Fig. 2. In the controls no effects were noted except for myocardial blood flow which was increased. When the two strains were analysed separately this finding was not observed.

Glucagon infusion caused a significant increase in blood flow in the spleen and small intestine. Pulmonary perfusion was significantly decreased. Histamine produced a decrease in the arterial blood flow of the liver as well as in the pulmonary blood

flow. Vasopressin infusion caused a decrease in all organs measured. Blood flow in the left and right kidney showed a correlation coefficient close to 1.00 both before and after infusion in all series.

The ratios between the tumor blood flow to surrounding liver tissue arterial blood flow are shown in Table II. The tumor to liver blood flow ratio decreased in the vasopressin infused rats. The other drugs tested, glucagon and histamine, showed no significant effects on tumor to liver blood flow ratio.

DISCUSSION

The vascular supply of hepatic tumors is predominantly of arterial origin. Ackerman has shown this in experimental tumors in rats (1972). He noticed that the arterial supply increased with the size of the tumor. In small tumors, less than 1–2 mm in diameter, supply from both the hepatic artery and portal vein was observed. As the tumors in our study were between 5–12 mm in diameter, nourishment was predominantly arterial. This is the reason why we have studied arterial blood flow to the tumor. The presented method is furthermore not well suited to measure portal flow to regional parts of the liver or intrahepatic tumor blood flow (Hafström et al., 1979c).

The effect of glucagon on hepatic blood flow has been studied in different species and the sum of the results indicates that liver blood flow increases in different species but there are differences depending on whether the increase is due to increased

Table II. Effects on relative tumor flow, calculated as tumor/arterial liver blood flow ratio after infusion of vasoactive drugs, mean $\pm$ S.E.M.

Drug	n	T/L ratio	
		Before	After
Saline	7	0.61 $\pm$ 0.25	1.34 $\pm$ 0.73
Glucagon	9	0.65 $\pm$ 0.18	0.93 $\pm$ 0.18
Histamine	9	0.47 $\pm$ 0.10	0.52 $\pm$ 0.15
Vasopressin	10	0.45 $\pm$ 0.09	0.25 $\pm$ 0.08*

* $p < 0.05$.

portal blood flow or increased arterial blood flow (Kock et al., 1970; Hulstaert et al., 1974; Krarup et al., 1974; Lindberg et al., 1976).

The effects of glucagon on arterial liver blood flow in the present study were not statistically significant but the blood flow of the spleen and the small intestine which reflects portal flow showed a significant increase thus indicating that there may be an increase in total liver blood flow induced by glucagon. This is in agreement with rat studies carried out by Ohnhaus (1972) using the ^{86}Rb rubidium method. He observed a 67% increase in total liver blood flow in rats. This might be due to an increased portal flow.

Blood flow in the liver tumors does not seem to be affected by glucagon as the tumor to liver arterial blood flow ratio was unchanged. This is contrary to the findings of Ackerman et al. (1977). Their studies did not, however, reflect the actual liver and tumor blood flow, as their experiments were designed to investigate the penetration of microfil into the tumors and they found that glucagon could promote this microfil penetration to the central part of the tumors.

Histamine has important vascular effects on man producing a sharp decrease in blood pressure and tachycardia. The cause is arteriolar dilation and increased capillary permeability and possibly also a constriction of small venules. This action varies in different species (Goth, 1978). Thus Ackerman 1973, using the Evans blue technique in rats, noticed an increased vascular permeability in the tumors after histamine injection.

In the present study there was no effect on the tumor to liver blood flow ratio indicating that the decrease in the tumor blood flow equalled the decrease in surrounding liver blood flow. These findings are not in agreement with another study by Ackerman (1972) who found that a decrease of the tumor to liver blood flow ratio occurred after histamine injection.

Vasopressin exerts profound action on circulation, which includes an arterial hypertension and a concomitant reflex bradycardia and a decreased cardiac output. Vasopressin induced a general vasoconstriction including the coronaries, hepatic arteries and the renal arteries. The hemodynamic effects of vasopressin have been extensively studied (Scaldon et al., 1960; Nylander, 1967; Schwartz, 1970; Ericsson, 1971; Greenway, 1972; Erwald et al., 1976; Gelman et al., 1978). A syn-

thetic vasopressin (lycin-8-vasopressin, Postacton[®] Ferring Co.) has been used in our experiments to study the relative effect on tumor blood flow in the liver. This substance has effects identical to those of normal vasopressin. There are no differences between species regarding specific reactions to vasopressin.

The registered effects of vasopressin infusion in the present study were that blood pressure increased significantly whereas heart rate and cardiac output decreased. The effect on different organs and tissues were that of a potent constrictor showing a significant decrease in the blood flow of all organs and tissues measured, including the tumor. In fact, tumor to liver blood flow ratio in the group of animals studied showed a significant decrease of tumor blood flow in relation to surrounding liver parenchyma and thus a more prominent constriction of the tumor than the liver vessels.

In summary, according to the findings in our experimental series of anaesthetized rats there was no significant effect on relative tumor blood flow only in the vasopressin infused rats. The effect was a decrease in the tumor blood flow in relation to surrounding liver tissue. It is doubtful that this effect could be of any value in the palliative treatment of liver cancer in man.

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REFERENCES

- Ackerman, N. B. 1972. Experimental studies on the circulatory dynamics of intrahepatic tumor blood supply. *Cancer* 29, 435.
- 1973. Differences in vascular permeability between normal and tumor vessels produced by vasoactive agents. *Surg Forum* 24, 105.
- Ackerman, N. B. & Hechmer, P. A. 1977. Effect of pharmacological agents on the microcirculation of tumors implanted in the liver. *Bibl Anat* 15, 301.
- Ericsson, B. F. 1971. Hemodynamic effects of vasopressin. *Acta Chir Scand*, Suppl 414, 4.
- Erwald, R., Wiechel, K.-L. & Strandell, T. 1976. Effects of vasopressin on regional splanchnic blood flows in conscious man. *Acta Chir Scand* 142, 36.
- Gelman, S. & Ernst, E. A. 1978. Nitroprusside prevents adverse hemodynamic effects of vasopressin. *Arch Surg* 113, 146.

- Goth, A. 1978. Medical pharmacology, 9th ed. The C. V. Mosby Company, Saint Louis.
- Greenway, C. V. & Murthy, V. S. 1972. Effects of vasopressin and isoprenalin infusions on the distribution of blood flow in the intestine; criteria for the validity of microsphere studies. *Br J Pharmacol* 46, 177.
- Hafström, L., Jönsson, P.-E., Landberg, T., Owman, T. & Sundqvist, K. 1979 a. Intraarterial infusion chemotherapy (5-FU) in patients with inextirpable or locally recurrent rectal cancer. *Am J Surg* 137, 757.
- Hafström, L., Nobin, A., Persson, B. & Sundqvist, K. 1979 b. Effects of catecholamines on cardiovascular response and blood flow distribution to normal tissue and experimental liver tumors. *Cancer Res* 40, 481.
- Hafström, L., Persson, B. & Sundqvist, K. 1979 c. Measurements of cardiac output and organ blood flow in rats using $^{99}\text{Tc}^m$ labelled microspheres. *Acta Physiol Scand* 106, 123.
- Hulstaert, P. F., Beijer, H. J. N. & Brouwer, S. A. S. 1974. Glucagon: hemodynamic action related to the effect of K^+ and Na^+ metabolism. *J Appl Physiol* 37, 556.
- Kock, N. G., Roding, B. & Hahnloser, P. 1970. The effect of glucagon on hepatic blood flow: an experimental study in the dog. *Arch Surg* 100, 147.
- Krarup, N. & Larsen, J. A. 1974. The effect of glucagon on hepato-splanchnic hemodynamics, functional capacity, and metabolism of the liver in cats. *Acta Physiol Scand* 91, 42.
- Krylova, N. W. 1969. Characteristics of microcirculation in experimental tumors. *Bibl Anat* 10, 301.
- Lindberg, B. & Darle, N. 1976. Effect of glucagon on hepatic circulation in the pig. *Arch Surg* 111, 1379.
- Nylander, G. 1967. Vascular response to vasopressin as reflected in angiography. An experimental study in the dog. *Acta Radiol*, Suppl. 266, 5.
- Ohnhaus, E. E. 1972. The effect of glucagon on the distribution of blood flow in the splanchnic area. *Life Sci* 11, 1155.
- Ong, G. B., Chan, P. K. W. & Alagaratnam, T. T. 1975. Clinical trials of inoperable primary carcinoma of the liver. *Bull Soc Int Chir* 5, 391.
- Schwartz, S. I. 1970. Influence of vasoactive drugs on portal circulation. *Ann N Y Acad Sci* 170, 296.
- Schaldon, S. & Sherlock, S. 1960. The use of vasopressin (Pitressin) in the control of bleeding from esophageal varices. *Lancet* 2, 222.
- Sundqvist, K., Hafström, L., Jönsson, P.-E., Rydén, S., Forsberg, L. & Lunderquist, A. 1978. Treatment of liver cancer with regional intraarterial 5-FU infusion. *Am J Surg* 136, 328.

